

REMARKS

The foregoing amendments and the following remarks are submitted in response to the communication dated December 30, 2005.

The foregoing amendments to page 12 and 102 of the Specification were made to correct an obvious error in the marking and recitation of the specific location and number of conservative and nonconservative amino acid differences among the amino acid residues of the mature mouse and human OB proteins. More specifically, at page 102, lines 17-20, the Specification as filed states that

“The N-termini of the mature proteins from both species share even higher homology, with only four conservative and three nonconservative amino acid substitutions among the N-terminal 100 amino acid residues.”

However, Figure 4, which provides an alignment and comparison of the mouse and human amino acid sequences, demonstrates that there are in fact six conservative and six nonconservative amino acid substitutions among the N-terminal 100 amino acid residues of the mature mouse and human OB polypeptides. In view of cleavage of the OB polypeptide at the end of the signal sequence, after amino acid Alanine 21 (as detailed in the Specification, including at page 71, lines 14-21), the “N-terminal 100 amino acid residues” of the mature proteins corresponds to amino acids 22-122, starting with V (Valine). The skilled artisan can readily recognize that the mouse and human sequences from 22-122 show six conservative amino acid substitutions (specifically, R-K at 56, S-T at 71, V-I at 85, L-M at 89, L-I at 95 and L-V at 110) and six nonconservative amino acid substitutions (specifically, A-S at 53, Q-R at 92, A-S at 98, S-H at 118, Q-W at 121 and T-A at 122). The Specification description of Figure 4 has similarly been corrected to properly reflect the accurate number of amino acid substitutions between the valine at codon 22 and the cysteine at position 117.

Applicants submit that this amendment does not introduce new matter into the specification because the correct conserved and nonconserved amino acid substitutions are readily determined by inspection of the sequences presented in Figure 4. Further, Applicants submit that a person of ordinary skill in the art, upon review of Figure 4 and the sequences

therein, would readily recognize the error and the correct number of conservative and nonconservative amino acid substitutions.

Status of the Claims

Claims 124, 132-137, 139-143, 145-153, 155-159 and 163-175 are now pending in the application. Claims 152 and 153 have been canceled without prejudice. Claims 124, 132-135, 139-143, 145-151, 155-159 and 163-173 have been amended and new claims 174 and 175 have been added in order to more particularly point out and distinctly claim that which Applicants regard as the invention. Support for the amended and new claims can be found generally through Applicants' Specification.

Claim Rejections – 35 USC § 112

Enablement

Claims 124, 132-137, 139-143, 145-149, 155-159 and 163-173 remain rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the Specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. The Examiner again sets out rejections as to the breadth of the claims, state of the art regarding the ob gene/protein, unpredictability of gene therapy and teachings of the Specification. Applicants respectfully disagree with the Examiner and assert that the Specification enables the skilled artisan to make and/or use the invention. Applicants acknowledge the Examiner's comments that the state of the art particularly regarding the OB protein and gene at the time of filing was limited – ob/ob mice were known to be obese, however the gene and protein corresponding to ob and their sequence were unknown – in fact, the isolation, cloning, sequencing, analysis and testing of the OB gene and protein are the subject of Applicant's invention. Applicants describe in the Specification the correlation of the now cloned OB gene to the ob mouse mutation, the sequence of OB from mouse and human, OB homologs evident in other mammals, the expression of OB mRNA in various mouse and human tissues (including adipose tissue and adipocytes), expression strategies

and successful expression of recombinant mouse and human OB protein, bioactivity of expressed OB recombinant protein, and biological effects of administration of OB to wild type and *ob/ob* mice. While certainly for instance, the art at filing may not teach what tissue expressed the OB protein, as asserted at page 4 of the Office Action by the Examiner, Applicant's Specification teaches this, and further, upon Applicant's disclosure of the OB sequence, the skilled artisan could readily and without undue experimentation determine the tissue(s) of OB expression.

Applicants again point out that, in fact, following the identification and disclosure of the OB sequence by Applicants, numerous groups have attempted and successfully achieved the administration of OB encoding vectors, the expression of OB polypeptide from these vectors, and correction or alteration of body weight *in vivo*. Applicants have previously provided published reports by several groups demonstrating OB/leptin gene therapy. As previously noted, Fletcher et al. (1996) and Muzzin et al. (1996) present studies showing the efficacy of *OB* gene therapy *in vivo* in *ob/ob* mice. Fletcher et al. (1996) also demonstrated *OB* gene therapy in wild-type mice. Each of these references used a different vector to achieve the therapeutic effect. Morsy (1998) also demonstrates weight loss following administration of leptin encoded adenoviral vector.

In asserting and arguing the unpredictability of gene therapy, the Examiner cites several reports which generally report some challenges in gene therapy. None of these citations, however, are particularly directed to leptin gene therapy and none of these citations report difficulties with leptin gene therapy. In fact, the leptin gene therapy references (Morsy, Muzzin, Fletcher) report successful expression of leptin from gene therapy vectors, whether transient or sustained.

Without question, there are a variety of viral vectors, of various origin and specificity, to choose from in undertaking gene therapy for expression of any therapeutic polypeptide. The selection and testing of these vectors is undertaken and can be accomplished by the skilled artisan. In addition, the skilled artisan will recognize that expressing a gene in arterial walls for treating restenosis is distinct from OB gene therapy and would not expect the results of Feldman (1995), for instance, to necessarily be relevant to OB therapy, where expression in arterial walls is not particularly targeted or appropriate.

Contrary to the Examiner's assertion that the instant Application does not provide enablement for the claims, Applicants continue to maintain that the Specification is a roadmap

for how one of skill in the art would modify the body weight of a mammal by administering to the mammal a vector comprising a nucleic acid molecule encoding an OB polypeptide that is capable of modulating body weight under conditions that provide for expression of the OB polypeptide *in vivo*. To this end, the present application discloses various vectors and compositions suitable for use in gene therapy applications. As previously detailed, the Specification at page 83 through 85 provides exemplary methods of introducing the *OB* gene *in vivo* using, for example, various viral vectors including retroviruses, adenoviruses, adeno-associated viruses as well as others. Such vectors were well known to those of skill in the art in 1994, and given the teaching of the present application, one of skill in the art would have been able to employ such vectors in the gene therapeutic applications of the present invention without undue experimentation. In fact, a number of research groups successfully did so, as evidenced by the Fletcher, Muzzin and Morsy references already provided and cited.

In further support of the ability of the skilled artisan to readily and successfully practice the claimed invention, Applicants provide herewith additional references as Exhibits A-E from distinct scientific research groups, who also report successful OB/leptin gene therapy studies. Chen et al (1996) (Exhibit A) report a 30% reduction in food intake and a virtual cessation of body weight gain over 28 days of study in hyperleptinemic rats, made hyperleptinemic by carotid artery infusion of recombinant adenovirus containing the rat leptin cDNA. In Exhibit B, Murphy et al (1997) report long-term correction of obesity and diabetes in genetically obese mice by a single intramuscular injection of recombinant adeno-associated virus encoding mouse leptin. Buettner et al (2000) (Exhibit C) demonstrate 40% decrease in adipose mass and normalized plasma glucose and insulin levels in high fat (HF) diet fed Wistar rats upon administration of recombinant adenovirus containing leptin. Dube et al (2002) (Exhibit D) use intracerebroventricular injection of recombinant adeno-associated virus encoding rat leptin in rats fed a high-fat diet (HFD) to reduce food intake and block the HFD-induced increase in weight, adiposity and metabolic variables (free fatty acids, triglycerides, insulin). Also, Larcher et al (2001) implement a cutaneous gene therapy approach, transplanting epithelial grafts of human keratinocytes transduced with a retroviral vector encoding leptin to induce a drop in blood glucose and food intake and body weight reduction in mice (Exhibit E). These references clearly confirm the feasibility of *OB* gene therapy methods to modify body weight in mice, humans and other mammals, as taught by the Specification, and confirm that the gene therapy

aspects of the present invention are enabled and can be practiced by the skilled artisan without undue experimentation.

Applicants disagree with the Examiner's position that a particular and specific combination of vector (retrovirus, adenovirus, etc.), specific type of administration (tail vein injection), and dosage is essential to the invention and particularly disagree that any such combination, for example adenoviral vector via the tail vein at a dosage of $1-2 \times 10^{11}$ (as described by Morsy), is an inventive or essential combination. It is not necessary or appropriate for the Applicants' instant Specification to teach and describe any and all such particular and specific combinations – it is well within the skill of the artisan to test and determine them. This is factually supported by the successful leptin gene therapy studies undertaken and reported by various groups, where distinct leptin genes, vectors, dosages and administration modalities are successfully implemented.

Applicants have provided specific examples of areas in which OB/leptin gene therapy has been shown to be successful. Thus, while there may be obstacles to the skilled artisan in various gene therapy methods, such obstacles are certainly not insurmountable, particularly in the case of OB/leptin gene therapy, and are navigable by those of skill in the art given that the instant application provides details of the gene sequences and vectors that can be used in such protocols in modulating body weight *in vivo*.

New Matter

Claims 124, 132-137, 139-143, 145-149, 155-159 and 163-173 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The Examiner asserts that the claims contain subject matter which was not described in the Specification so as to reasonably convey to the skilled artisan that the inventors had possession of the claimed invention at the time the Application was filed. Applicants disagree with the Examiner's assertion and rejection.

The Examiner again asserts that the phrase “conditions that provide for expression of the OB polypeptide *in vivo*” in claims 124, 132-135, 163-165 and 167 is new matter. Again, Applicants respectfully disagree. Applicants further submit that the above claim amendments, in which the language is modified to refer to a “sequence encoding OB polypeptide operatively linked to a promoter” and “administered in a therapeutically effective amount”, render this rejection moot and are clearly supported by language in the Specification, including at page 51, lines 16-25, page 52, lines 9-17, page 53, lines 9-11 and at page 72, line 25 through page 73, line 2, respectively.

The phrase “operatively associated with an expression control sequence” in claims 139-143, 145-149 and 155-157 is again rejected as new matter. Applicants disagree and submit that, in fact, the Specification provides clear support for this phrase. Applicants have above, however, modified the language of the claims to refer to “operatively linked to a promoter” which is supported by the Specification, including at page 51, lines 16-25, page 52, lines 9-17, and page 53, lines 9-11.

The Examiner again rejects “83 percent or more amino acid identity to the OB polypeptide amino acid sequence set out in SEQ ID NOs: 2, 4, 5, 6, 23 or 25” in claims 133 and 147 as new matter. Applicants again disagree and point out that the Specification, including in Figure 4, the description at page 12, lines 15-24, with reference to Figure 4, and page 102, lines 15-20, provides specific support and recitation of 83% identity at the amino acid level. Support for the term “83% or greater amino acid identity”, is found in the comparison of the disclosed amino acid sequences of mouse and human OB polypeptides. Specifically the Specification now properly characterizes the percentage (%) identity between the mouse and human OB amino acid sequences, where at page 12, lines 20-21, the Specification now states that

“Overall, there is 83% identity at the amino acid level, ...”

and at page 102, lines 15-17, the specification now states that

“Comparison of the human and mouse ob polypeptide sequences showed that the two molecules share an overall 83% identity at the amino acid level (Figure 4).”

In addition, Figure 4, which provides a comparison of the mouse and human amino acid sequences aligned with one another, demonstrates that there are 28 amino acid differences between the human and mouse sequences, out of a total of 167 amino acids. Thus, 139 amino

acids, or 83%, are identical, i.e. Alanine for Alanine, Glutamine for Glutamine. It is believed to be particularly straightforward for the skilled artisan to make such a comparison since the length of the mouse and human OB polypeptides are the same and the corresponding amino acids to compare in assessing identity is very readily determined -no gaps exist and no accounting for regions of extra amino acids is necessary. In addition, the skilled artisan's comparison of SEQ ID NO: 5 (which is the mouse variant OB polypeptide with glutamine 49 deleted) with SEQ ID NO:6 (which is the human variant OB polypeptide with glutamine 49 deleted) will yield the same result of 83% amino acid identity in a similarly straightforward fashion.

The Examiner again asserts that the concept of an OB protein comprising "amino acids 22-167 of SEQ ID NO: 4 wherein one or more amino acids selected from the group consisting of amino acids ... 56 ... [and] ... 95 ... is substituted with another amino acid" in claims 134, 142, 148 and 158 is new matter. In addition, the concept of an OB protein comprising "amino acids 22-166 of SEQ ID NO: 6 wherein one or more amino acids selected from the group consisting of amino acids 52, 55, 70 ... 162 and 165 is substituted with another amino acid" in claims 135, 143, 149 and 159 is again rejected as new matter. Applicants respectfully disagree and submit that the Specification has clear support for this claim language and for the substitution of these particular amino acids with another amino acid. The Specification anticipates variants of OB polypeptides wherein amino acids are substituted. These rejected claims refer particularly to OB polypeptides wherein another amino acid is substituted at any particular and specified site(s) where the amino acids present in the disclosed mouse and human OB polypeptides are different. These amino acid substitution sites are clearly evident from Figure 4, which sets out a comparison of the mouse and human amino acid sequences aligned with one another. In Figure 4, those specific amino acids which are different between the human and mouse sequences are noted and can be readily identified by the skilled artisan. These specific amino acids are those listed to be selected from in the rejected claims 134, 142, 148, 158, 135, 143, 149 and 159. It is believed to be particularly straightforward for the skilled artisan to make such a comparison since the length of the mouse and human OB polypeptides are the same and the corresponding amino acids to compare in assessing differences is very readily determined - no gaps exist and no accounting for regions of extra amino acids is necessary. Figure 1 depicts mouse (murine) amino acid sequence and Figure 3 depicts human sequence. These sequences, each 167 amino acids, are compared in Figure 4 and the particular amino acids where they differ and substitutions at

these amino acids are listed in claims 134, 142, 148 and 158. Mutant or variant - gln deletion at amino acid 49 - OB sequences are depicted for mouse in Figure 5 and human in Figure 6. Each of these deletion mutant OB polypeptides are 166 amino acids, having a single amino acid deleted (gln 49). Therefore, the amino acid sequence numbering for the gln mutants is one less than the numbering in wild type OB for each of amino acids 50-167 (corresponding in the mutant to amino acids 49 through 166). The particular amino acids where the mouse and human gln mutants differ and substitutions at these amino acids are listed in claims 135, 143, 149 and 159.

The Examiner further rejects the concept of administering viral vectors by infection or liposome mediated transfection in claim 151 as new matter. Applicants disagree and point to the Specification at page 84, lines 1-16, for description and support for this concept. Pages 83-84 of the Specification discuss various vectors for and under the heading "Nucleic Acid-based Therapeutic Treatment", where the section begins stating "The ob gene can be introduced ... to develop gene therapy for obesity." Various vectors are then discussed and at page 84, line 13, the Specification clearly states:

"Alternatively, the vector can be introduced *in vivo* by lipofection."

Applicants cannot agree with the Examiner in his assertion that 'nowhere does the section on nucleic acid-based therapeutic treatments contemplate administering the viral vectors by infection or lipofection'. Applicants, however, have above made amendments to claim 151 wherein the language of the claim is altered and assert that this rejection is now moot.

Lastly, the Examiner again asserts that claims 165-173, reciting particular OB polypeptide analogs, are new matter and that support cannot be found in the Specification. Applicants disagree and point to section in the Specification entitled "Analog of the Ob Polypeptide", including pages 32-35, where it discusses and provides support for the various amino acid substitutions, and amino acid deletions (truncated analogs) set out in these claims. This portion of the Specification sets out each of the substitutions and truncated analogs claimed. At page 32, line 26 through page 33, line 10, it is stated:

For example, the serine residue at position 53 or position 98, or both (in the unprocessed peptide sequence depicted in Figure 4) from human may be substituted, *e.g.*, with glycine, alanine, valine, cysteine, methionine, or threonine. Similarly, the arginine residue at position number 92 (Figure 4) may be substituted, *e.g.*, with asparagine, lysine, histidine, glutamine, glutamic acid, aspartic acid, serine, threonine, methionine, or cysteine. Referring still to Figure 4, other amino acids in the human OB peptide that appear to be capable of

substitution are lysine at position 56, threonine at position 71, isoleucine at position 85, methionine at position 89, isoleucine at position 95, valine at position 110, histidine at position 118, tryptophan at position 121, alanine at position 122, glutamic acid at position 126, threonine at position 127, leucine at position 128, aspartic acid at position 129, glycine at position 132, glycine at position 139, methionine at position 157, tryptophan at position 159, leucine at position 163, and glycine at position 166. In another embodiment, it may be possible to substitute one or more of residues 121 to 128 (as depicted in Figure 4), *e.g.*, with glycines or alanines, or substituting some of the residues with the exceptions of serine as position 123, or leucine at position 125.

This above language supports each of claims 165, 166, 170, and 171. Claims 169 and 172 are supported in the Specification's next paragraph beginning at page 33, line 11 and continuing to page 34. The N-terminal amino acids set out in claims 167, 168, and 173, and recited in subparts h) of claims 169 and 172 are supported in the Specification, including in Figures 21 and 22, in the Figure legends at page 18 and in Example 6, which details expression and various constructs in yeast and using His tags. Each of these N terminal amino acid sequences are depicted and described and they therefore do not constitute new matter as set out in the claims.

Indefiniteness

The Examiner has rejected claim 140 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter applicant regards as the invention. The Examiner again rejects the phrase "such OB encoding DNA" in claim 140 as lacking antecedent basis. Applicants have above amended claim 140 and assert that claim 140 as amended is definite.

In view of the foregoing amendments and remarks, Applicants submit that each and all of the Examiner's enablement, new matter and indefiniteness rejections under 35 USC 112, second paragraph, should be withdrawn.

CONCLUSION

Applicants respectfully request entry of the foregoing amendments and remarks in the file history of the instant Application. The Claims as amended are believed to be in condition for allowance, and reconsideration and withdrawal of all of the outstanding rejections is therefore believed in order. Early and favorable action on the claims is earnestly solicited.

Respectfully submitted,

A handwritten signature in black ink, appearing to read 'Christine E. Dietzel', written in a cursive style.

Christine E. Dietzel, Ph.D.
Agent for Applicants
Registration No. 37,309

KLAUBER & JACKSON
411 Hackensack Avenue
Hackensack, NJ 07601
(201) 487-5800